Microbiology of Ulcer

8

Gopal Nath and Gopal Kumar

8.1 Introduction

The primary function of normal, intact skin is to control microbial populations that reside on the skin surface and to prevent underlying tissue from becoming colonized and invaded by potential pathogens. Ulcer causes break in continuity of skin and makes a path for entry of pathogen. Infections of the lower extremity ulcers are a major source of morbidity and important cause of amputation and sometimes mortality in patients particularly with neuropathy and diabetes. Not all ulcers are infected. Evaluation of infection should involve a thorough examination of the extremity for clinical signs of infection along with appropriate laboratory and imaging studies. The organisms implicated are often *Staphylococcus aureus* (often MRSA in diabetic patients) and Group B *streptococci* in limb-threatening infection. Chronic infected wounds often have multidrug-resistant pathogens, especially after exposure to health care procedures and use of multiple antibiotics. The presence of a foot ulcer should heighten the index of suspicion for associated infection.

8.2 Microbiology

8.2.1 The Normal Microbiota

The skin and mucus membranes of human beings always harbor a diverse number of microorganisms that can be broadly divided into two major groups:

The *resident flora* which consists of relatively fixed types of microorganisms regularly found in a given area at a given age, and if disturbed anyhow, it

G. Nath (✉) • G. Kumar
Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India
e-mail: gopalnath@gmail.com; gopalvanvani@gmail.com

A.K. Khanna, S.K. Tiwary (eds.), *Ulcers of the Lower Extremity*,
DOI 10.1007/978-81-322-2635-2_8

reestablishes itself. The *transient flora* consists of nonpathogenic or potentially pathogenic microorganisms that inhabit the skin or mucous membranes for hours to weeks; these are derived from the environment, do not produce any disease, and are not able to establish permanently on the surface. Members of the transient flora are generally of little significance so long as the normal resident flora remains intact. However, transient microorganisms may colonize, may proliferate, and may produce disease if the resident flora is disturbed.

It is likely that the culturable microorganisms in the laboratory represent only a fraction of those that are part of the normal resident or transient microbial flora of the area. When a range of polymerase chain reaction is used to amplify bacterial 16SrDNA, many previously unidentified bacteria can be detected. The number of species that make up the normal micro biota has been shown to be much greater than is recognized. Thus, the understanding and identification of normal micro biota is still in transition.

The microorganisms that are constantly present on the body surfaces are commensals, and their presence in that particular area depends upon many factors such as temperature, moisture, as well as the presence of certain nutrients and inhibitory factors. On mucous membrane and the skin, the resident flora may prevent colonization by pathogens and possible disease through bacterial interference which may involve competition for nutrients, mutual inhibition by metabolic or toxic products, and mutual inhibition by antibiotics or bacteriocins.

8.3 Role of Host and Environment

The most important factor in limiting the infection is the host resistance. Suppression of the normal microbiota creates a partial local gap that tends to be filled by organisms from the environment or from other parts of the body. Such organisms behave as opportunists and often become pathogens when conditions favor.

On the other hand, members of the normal micro biota may themselves produce disease under certain circumstances. These organisms get adapted to the noninvasive mode of life defined by the limitations of the surrounding environment. If forcefully removed from the restrictions of that environment and introduced into the bloodstream or tissues, these organisms may become pathogenic. The surrounding environment causes constant exposure of skin with different microbes, and so the skin is apt to contain transient microbiota. The constant and well-defined resident flora is modified in different anatomic areas by secretions, habitual wearing of clothing, or proximity to mucous membranes.

8.4 Role of Microbes in Infection

Most acute and chronic wound infections involve mixed population of both aerobic and anaerobic microorganisms. The predominant resident microorganisms of the skin are aerobic and anaerobic diphtheroid bacilli (e.g., *Corynebacterium,*

Propionibacterium); nonhemolytic aerobic and anaerobic staphylococci (*S. epidermidis* and other coagulase-negative staphylococci, occasionally *S. aureus* and *Peptostreptococcus* species); Gram-positive, aerobic, spore-forming bacilli that are ubiquitous in air, water, and soil; alpha hemolytic *Streptococci* (viridians streptococci) and Enterococci, and Gram-negative coliform bacilli and *Acinetobacter*. Fungi and yeast are often present in skin folds; acid-fast nonpathogenic mycobacteria occur in areas rich in sebaceous secretions (genitalia, external ear). The number of superficial microorganisms may be diminished by vigorous daily scrubbing with soap containing hexachlorophene or other disinfectants, but the flora is rapidly replenished from sebaceous and sweat glands.

Anaerobic and aerobic bacteria often join to form synergistic infections (gangrene, necrotizing fasciitis, and cellulitis) of the skin and soft tissue. These bacteria are frequently part of normal microbial flora. So mixture of microorganisms is usually involved in many skin lesions.

8.5 The Role of Biofilm in Infected Ulcer

Most chronic ulcers are colonized by bacteria in the form of a biofilm, which is difficult to treat [1]. Although the adverse effect of bacteria on wound healing has long been noted, biofilm, which does not always give rise to an overt phenotype or infectious picture, has been underappreciated. Indeed, a nonhealing wound is one of the more common presentations of biofilm.

Biofilm consists of a sessile community of multiple bacterial species enclosed by a protective carbohydrate-rich polymeric matrix that is resistant to antimicrobial and immune cell penetration [2]. Most wounds are in fact colonized by bacteria that set up in the form of biofilm. Unfortunately biofilm is exceedingly tenacious and readily accumulates after debridement. Thus, proper dressing care consists of dressings that both treat the wound and minimize biofilm accumulation.

Bacteria whether free floating or incorporated within a biofilm are extremely detrimental to wound healing, particularly when they reach the level of critical colonization [3].

8.6 Bioload of Ulcer Wound

Wounds may be classified as contaminated, colonized, critically colonized, or infected [4]. This classification is useful to understand relation between the bacteria and the patient (or host) and define the level of bioburden (i.e., the cost exacted by bacteria from the resources of the wound and the patient). All wounds are contaminated to some degree either by skin flora or by environmental pathogens.

It is likely that this level of bacterial contamination stimulates wound repair mechanisms by upregulating the inflammatory response. When the contaminating bacteria begin to proliferate, the wound is said to be colonized; however, when the

wound provokes an inflammatory reaction by the proliferating bacteria, the wound is said to be infected. It is important to keep in mind both host inflammatory reactions contribute to failure in wound healing as bacteria themselves do [5].

Microbiological factors such as the population density, the types of microorganisms present, and the microbial interactions and host factors such as the efficacy of the immune response and the condition of the tissue are all critical and must be considered collectively as factors predisposing to infection.

Antimicrobial treatment of clinically infected and/or nonhealing polymicrobial wounds should cover a variety of potentially synergistic aerobic or facultative and anaerobic microorganisms and should not simply target specific pathogens (e.g., *S. aureus and P. aeruginosa*).

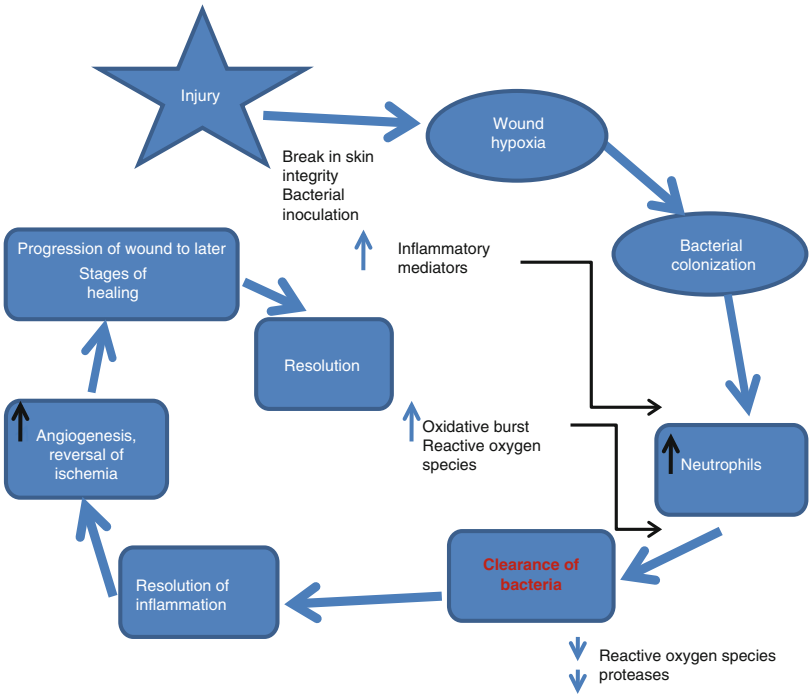
Judicious use of antibiotics, adequate debridement, and proper dressing choices can decrease bacterial numbers and reduce the competition for resources occurring in wounds contaminated by bacteria [3].

8.7 Vicious Cycle of Wound Healing

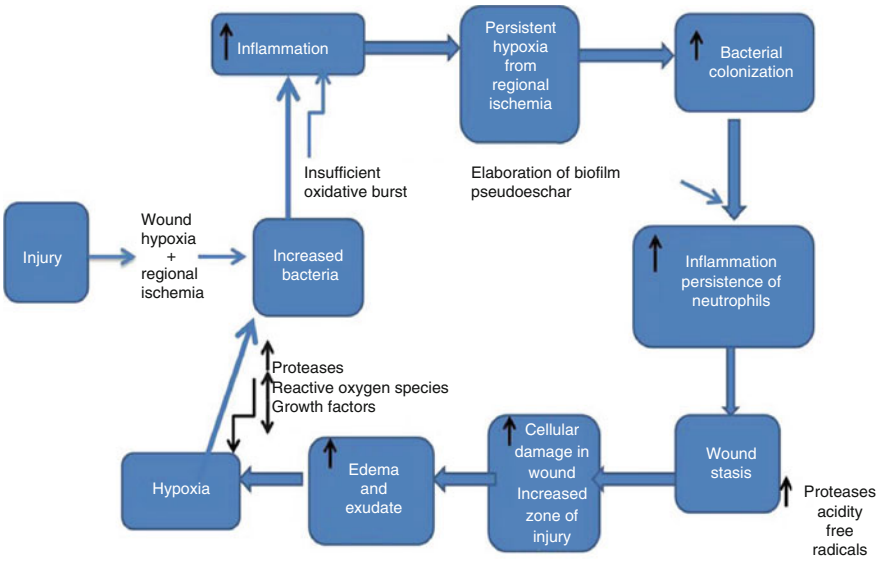
Schematic representation depicts interplay between bacterial levels, oxidative stress, and parameters of healing in a wound:

Lower extremity ulcers, *ACS Surgery* [42]

A. Typical self-limiting inflammatory response in a healthy healing wound:



B. Impaired healing of wound:



8.8 Infective Causes of Ulcer

8.8.1 Infectious Causes of Limb Ulcers

Ulceration in the lower extremity may be due to infectious agents. Diverse groups of microbes including bacteria, viruses, parasites, and fungi have been implicated [41].

Disease	Causative agent
Erysipelas (bullosa)	*Streptococcus pyogenes*
Fasciitis necroticans	*Streptococcus hemolyticus*
Ulcerating pyoderma	*Staphylococcus aureus*
Ecthyma gangrenosum	*Pseudomonas spp.*
Gas gangrene	*Clostridium spp.*
Septic embolism	*Meningococcus* and others
Anthrax	*Bacillus anthracis*
Diphtheria	*Corynebacterium diphtheriae*
Osteomyelitis	Several microorganisms specially *Staphylococcus aureus*
Herpes, CMV	HSV, CMV
Lues maligna (malignant syphilis)	*Treponema pallidum*
Tularemia	*Francisella tularensis*
Tropical ulcer	*Bacteroides, Borrelia vincentii,* and other bacteria
Maduromycosis (eumycetoma/ mycetoma)	*Nocardia brasiliensis, Exophiala jeanselmei*
Chromoblastomycosis, coccidioidomycosis, sporotrichosis, granuloma	Several bacteria; *Coccidioides immitis* or *Coccidioides posadasii*; *Sporothrix schenckii*; dermatophytes of the genera Trichophyton and Microsporum

Disease	Causative agent
Histoplasmosis	*Histoplasma capsulatum*
Buruli ulcer	*Mycobacterium ulcerans*
Bacillary angiomatosis	*Bartonella henselae* or *Bartonella quintana*
Ulcerating cutaneous tuberculosis	*Mycobacterium tuberculosis*
Amebiasis	*Entamoeba histolytica, Acanthamoeba*
Leishmaniasis	*Leishmania donovani* complex, *Leishmania mexicana* complex, *Leishmania tropica*; Leishmania major; *Leishmania aethiopica*
Leprosy	*Mycobacterium leprae and Mycobacterium lepromatosis*

CMV cytomegalovirus, *HSV* herpes simplex virus

8.9 Infected Ulcers

Some microorganisms can cause tissue necrosis, such as the notorious Group A β-hemolytic *Streptococcus pyogenes.* These bacteria have been implicated into a range of severe clinical symptoms varying from erysipelas, ecthyma, and fasciitis necroticans to deep cellulitis, sepsis, and multiorgan failure.

Almost all chronic wounds are secondarily contaminated with bacteria, but in most cases, with the exception of few, they are not of pathogenetic importance. Wound swab cultures are often routinely performed, but give only information about the bacterial flora in the superficial layers. The decision to prescribe systemic antibiotics should be based on the combination of culture results and clinical criteria, such as signs of infection (fever, erythema, calor).

Acquired immune deficiency due to human immunodeficiency virus (HIV) infection reintroduced ulcerative conditions that were thought to be eradicated, such as tertiary lues and ulcerating tuberculosis, and may be associated with atypical, large ulcers caused by herpes simplex or cytomegalovirus. In addition, bacillary angiomatosis, caused by *Rochalimaea* species, and *Histoplasma capsulatum* must be included in the differential diagnosis of ulcerations occurring in HIV disease [6, 7]. Increased world travel has brought tropical ulcerating infections to Western countries, especially leishmaniasis, but also atypical mycobacteria, ulcus tropicum [8], and deep mycotic infections.

Tuberculous cutaneous ulcer might occur in erythema induratum or Bazin's disease, situated usually on the back of the calves [9].

Ulcer by amoeba: Ulceration of the skin of the lower limbs by amoebae, which could be the result of superinfections of skin wounds due to scratching with dirty nails.

Tropical ulcer: These are necrotic painful lesions that result from a mixed bacterial infection. They are common in hot humid tropical or subtropical areas, where they occur on the lower legs or feet of children and young adults.

8.9.1 Other Infective Causes of Ulcer

Cutaneous tuberculosis
Syphilis

Parasitic infection
Fungal infection

8.10 Infected Diabetic Foot Ulcers

In most cases, diabetic foot infections are polymicrobial, and deep tissue culture after debridement is essential for identifying the true pathogens. Diabetic foot infections are frequently associated with *S. aureus, epidermidis, Streptococcus spp., P. aeruginosa, Enterococcus spp.,* and coliform bacteria [10].

8.10.1 Infection Status of Chronic Wounds

Chronic leg ulcers are defined as those that show no tendency to heal after 3 months of appropriate treatment or are still not fully healed at 12 months.

The interaction between ulcer and bacteria can be stratified into four levels: contamination, colonization, critical colonization, and infection [11], while contamination and colonization by microbes are not believed to inhibit healing, the line between colonization and infection can be difficult to define.

The term "critical colonization" has been used to describe the stage at which bacteria begin to adversely affect wound healing [11]. Moreover, the underlying pathogenesis of chronic wounds may result in wounds of different etiologies being differently affected by bacteria [12–14].

Chronic wounds by their very nature may not always display the classic symptoms of infection (pain, erythema, edema, heat, and purulence), and it has been suggested that an expanded list, including signs specific to secondary wounds (such as serous exudate plus concurrent inflammation, delayed healing, color of granulation tissue, foul odor, and wound breakdown) be employed to identify infection [15].

Microbiologically, a critical bacterial load, synergic relationships between bacterial species, and the presence of specific pathogens have all been proposed as indicators of infection. The presence of microbes per se is not indicative of wound infection.

8.10.2 Microbial Load and Healing of Wound

The possibility that a critical microbial load might directly affect the healing outcome in both acute and chronic wounds has been considered for several decades, with a direct relationship first being demonstrated by Bendy et al. [16] in 1964. Since then, work carried out by Robson [17] and others has led to the widely held opinion that nonhealing is associated with a bacterial load of more than 10^5 bacteria per gram of tissue.

The concept of bacterial synergy which recognizes the importance of interspecies interactions has been purported to occur in chronic wounds through studies such as that by Bowler and Davies [18]. They found the growth and pigmentation of

some Gram-negative anaerobes to be enhanced by some facultative bacteria through the provision of an essential, unidentified growth factor. Furthermore, they found significantly greater numbers of anaerobes in infected ulcers compared with noninfected ones.

With regard to specific pathogens, beta-hemolytic *streptococci* [17, 19] *S. aureus* [12], Enterobacteriaceae [12], and *Pseudomonas* species [12, 20] have all been implicated as having potentially adverse effects on wound healing. The impact of these species may vary in different settings, for example, over 60 % of arterial and diabetic ulcers colonized with *S. aureus* develop an infection compared with only 20 % of venous ulcers similarly colonized [12].

In summary, microorganisms are identified in the deep tissue of all chronic wounds, yet the role they play and the impact of specific species on wound longevity are not clear. The distinction between infected and colonized wounds has to be considered on a clinical basis and not by microbiological analysis only due to the universal colonization of chronic wounds [21]. Microbial analysis can be of benefit when considered in concert with clinical observations to confirm causative organisms and their sensitivities [22] and so enable refinement of antibiotic regimens [21].

8.10.3 Microbiology, Antibiotic Usage, and Resistance in Leg Ulcers

The microflora of leg and foot ulcers is usually polymicrobial, and recent studies using molecular techniques have emphasized the complex ecology of these wounds [23, 24]. Using conventional techniques, the mean number of bacterial species per ulcer has been found to range from 1.6 up to 4.4 [25–28]. Hansson et al. [29] observed that 86 % of ulcers with no clinical signs of infection contained more than one bacterial species.

Staphylococcus aureus and coagulase-negative *Staphylococcus* have been the predominant organisms isolated. *S. aureus* has been reported in frequencies varying from 43 % of infected leg ulcers to 88 % of noninfected leg ulcers [29], whereas *Staphylococcus epidermidis* has been reported in 14 % of venous ulcer specimens [30] and 20.6 % of diabetic foot ulcers (DFUs) [27]. *Pseudomonas aeruginosa* is another frequently identified organism and has been found in 7–33 % of ulcers [12, 26, 29]. A number of other aerobic species have also been reported, including *Escherichia coli* [18, 27, 29–31], *Enterobacter cloacae*, *Klebsiella* species, *Streptococcus* species, *Enterococcus* species [28, 29] and *Proteus* species [31]. This is by no means an exhaustive list, but is illustrative of the range of aerobic bacteria that exist in chronic wounds.

In addition to aerobes, anaerobic organisms are frequently identified in wounds, albeit with considerable variation. Trengove et al. [20] found obligate anaerobes in one-quarter of chronic leg ulcer samples, while Ge et al. [31] found they constituted only 6 % of DFU wound isolates.

However, a focused study by Bowler and Davies [18] found anaerobes in 73 % of noninfected leg ulcers and 82 % of infected leg ulcers. The most common isolates found in both the infected and noninfected leg ulcers were Peptostreptococcus species and pigmented and nonpigmented *Prevotella/Porphyromonas* species [18].

Finegoldia magna (previously classified as *Peptostreptococcus magnus*) was found by Hansson et al. [29] to be present in 19.6 % and *Peptoniphilus asaccharolyticus* in 9.8 % of noninfected venous leg ulcers. Kontiainen and Rinne [28] found that clinical swabs sent for analysis, presumably from infected or assumed infected wounds, yielded obligate anaerobic rods (mainly *Bacteroides* species) from 12 % of ulcers and anaerobic cocci (*Peptostreptococcus)* from 8 %. Ge et al. [31] found *Bacteroides, Peptostreptococcus,* and *Prevotella* species to be the most frequently isolated obligate anaerobes in mild or moderately infected DFUs. The continuity of the microbial profile of chronic wounds over time is unclear from the limited literature that has examined this issue. Hansson et al. [29] considered the microflora of chronic wounds to be a relatively stable entity having found that 90 % of ulcers that were followed for 4 months, or until healing, contained at least one resident organism that was isolated from all monthly swabs. Furthermore, Gilchrist and Reed [32] considered chronic wounds to have stable microbial populations, following the observation that once a species was present, it generally remained so under hydrocolloid dressings, with the exception of the transient appearance of *P. aeruginosa*. However, closer examination of their data shows that 85 % of wounds acquired new aerobes and 45 % new anaerobes over the 8 week of study period. Trengove et al. [20] logged the occurrence of new bacterial groups appearing in wounds after initial swabs had been taken. They found at least one new bacterial group present in subsequent swabs in 82 % of patients and thus concluded that the microbial populations of chronic wounds alter over time.

Each of these studies suggests that although there may be a degree of stability for some microbial populations, the chronic wound appears to be a dynamic environment. However, there are to date no definitive studies of bacterial succession within chronic wounds, the influence of antibiotics on this succession, or of the interactions between bacterial succession and healing.

8.11 Lab Diagnosis of Infected Ulcers

8.11.1 The Role of Microbiology in Diagnosis

The diagnosis of infected ulcer is made clinically and supported by microbiology lab reports. Finding purulent drainage (pus) or two or more signs or symptoms of inflammation, e.g., erythema, induration, swelling, pain, tenderness, or warmth, is indicative of infection.

Elevated concentration of C-reactive protein and procalcitonin can help distinguish mild or moderately infected ulcer wound from those that are uninfected [33].

Serologic tests for syphilis or polymerase chain reaction for mycobacterium DNA may be performed on specimens from an ulcer suspected of being of mycobacterial origin (e.g., erythema induratum of Bazin: Mycobacterial panniculitis with subsequent ulceration usually involving the calves). Cryoglobulins may be associated with hepatitis C and leg ulceration.

8.12 Culture

For culture, specimens should be obtained after the surface of the wound has been washed thoroughly by sterile saline and followed by debridement of superficial exudates. Specimens must be obtained by scraping the ulcer base or the deep portion of the wound edge with a sterile curette. A curettage, or tissue scraping with a scalpel, from the base of a debrided ulcer provides more accurate results than does the wound swab (Fig. 8.1).

Culture of open foot and leg ulcers cannot be used reliably to establish the presence of infection. These ulcers whether infected or not will contain multiple commensal or colonizing bacteria, some of which have the potential to become invasive pathogens.

Culturing specimens from a chronic wound that is healing at an expected rate and does not display any signs or symptoms of infection are unnecessary. Because all wounds are contaminated and colonized, a culture simply confirms the presence of microorganisms without providing any information as to whether they are having a detrimental effect on the host.

However, bacterial swabs can provide information on the predominant flora within a nonprogressing, deteriorating, or heavily exudating wound. Microbiological tests also can screen for multiresistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE).

The soft tissue specimens should be promptly sent to the laboratory and processed for aerobic and anaerobic bacteria.

Following incubation under aerobic or anaerobic conditions for 24–48 h, qualitative and semiquantitative assessments of the cultures are normally made. A minimally inflamed but deep ulceration may be associated with underlying osteomyelitis [34] (Fig. 8.2).

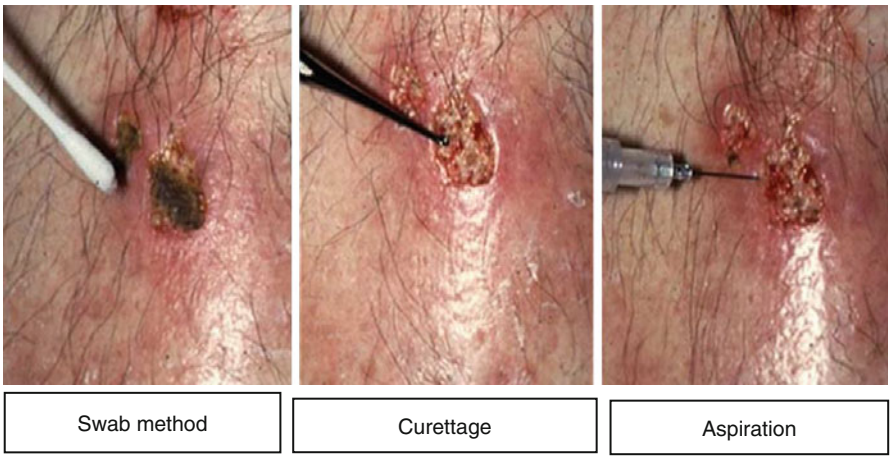


Fig. 8.1 Collection of specimen from ulcer

Fig. 8.2 McIntosh and Field's anaerobic jar

8.12.1 Picture of Common Isolates (Figs. 8.3–8.5)

Fig. 8.3 *Staphylococcus aureus* colonies on blood agar

Fig. 8.4 *Pseudomonas colonies* on nutrient agar

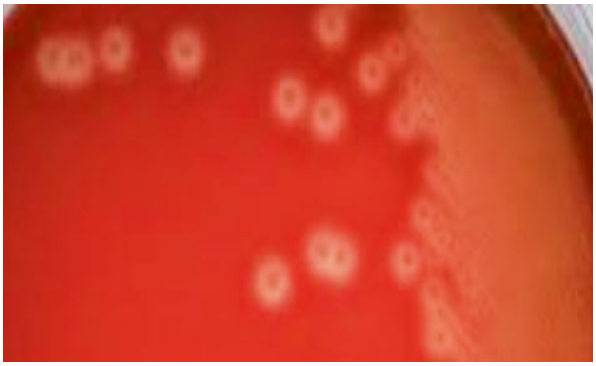


Fig. 8.5 Beta-hemolytic colonies of *Streptococcus pyogenes*

8.13 Gram Stain

The degree of inflammatory response is measured by the presence and quantity of neutrophils per high power field in the Gram stain of the swab contents before inoculating the specimen on growth media.

In case of cutaneous anthrax, a Gram stain smear of the lesion shows the typical, large Gram-positive rods (1–1.5 × 4–10 μm). The bacterium is noticeably larger than most other pathogens. An alternative to Gram stain is polychrome methylene blue (M'Fadyean's stain).

8.13.1 Which Culture Technique Should Be Used?

Quantitative sampling (tissue biopsy) has merits, and a strong association exists between the number of organisms in a wound and the ability of the wound to heal. Once bacterial load reaches 10^6 CFU/g of tissue, wound healing is usually impaired [35]. However, these findings need to be viewed in perspective. At least 20 % of wounds colonized with more than 10^5 CFU/g of tissue will still heal [36], and normal skin flora present in high quantities appears to enhance wound healing [37]. On the other hand, some microorganisms (e.g., beta-hemolytic Streptococci, *Mycobacterium tuberculosis, Treponema pallidum, Corynebacterium diphtheriae, Bacillus anthracis, Francisella spp.,* and *Brucella spp.*) can be detrimental in small numbers. Thus, quantitative microbiology does not necessarily provide an unambiguous diagnosis of infection (Figs. 8.3, 8.4, 8.5, 8.6, 8.7, and 8.8).

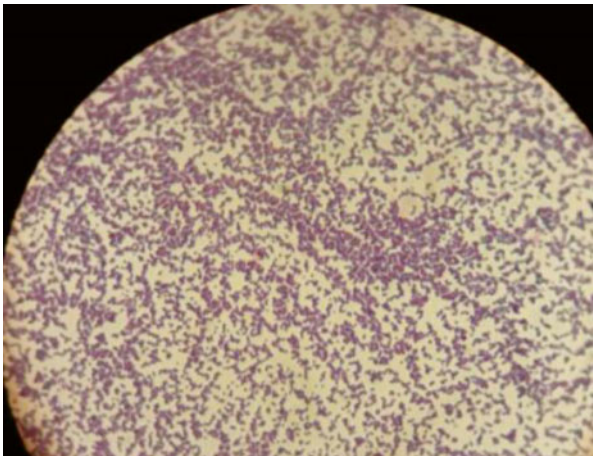


Fig. 8.6 Gram-positive cocci in clusters (*Staphylococcus aureus*)

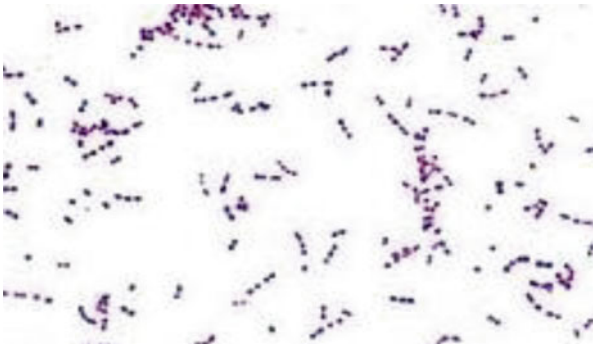


Fig. 8.7 Gram-positive cocci in short chain (*Streptococcus pyogenes*)

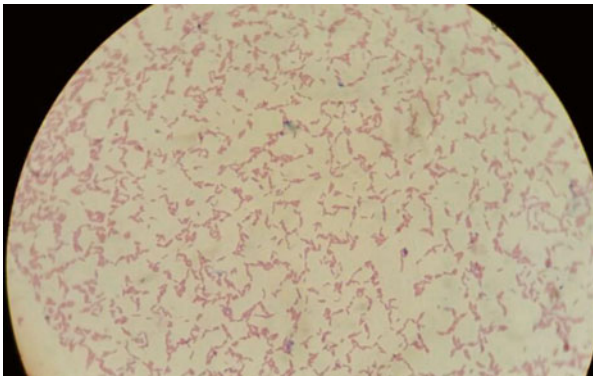


Fig. 8.8 Gram-negative bacilli (pseudomonas) on Gram stain

Qualitative aspects are at least as important as overall bacterial load, and evidence is growing that microbiology obtained by a swab may adequately correlate with qualitative findings obtained through tissue biopsy.

In a study on diabetic foot infections, Wheat et al. [38] showed that the results obtained with swabs are similar to those obtained with tissue biopsy.

Sapico et al. [39] found similar results in a study on chronic pressure ulcers, demonstrating a 75 % concordance between swab and biopsy results. Ehrenkranz et al. [40] demonstrated that an irrigation-aspiration technique could produce similar results to qualitative biopsy.

8.13.2 Procedure for Taking a Swab

In most cases, wounds should not be cultured if no evidence of infection or impaired healing is noted unless screening is being performed for colonization of multiresistant organisms. The wound bed must first be cleaned with saline and superficially debrided so the cultures from the superficial wound compartment more closely resemble those in the deep wound compartment.

Alginate or rayon-tipped swabs are sometimes preferred in the belief that the fatty acids contained in cotton swabs might inhibit growth in certain bacteria. However, the organisms commonly encountered in infection are likely to withstand the environment of a cotton swab (Fig. 8.9).

Pre-moistening a swab (Fig. 8.10) in the transport media is useful if the surface of the wound is dry but is not necessary if the wound is already moist. The swab should be taken from the surface of the granulation tissue wound. The tip of the swab should be rolled on its side for one full rotation over the part of the wound granulation tissue with the most obvious signs of infection, avoiding slough and surface purulent discharge.

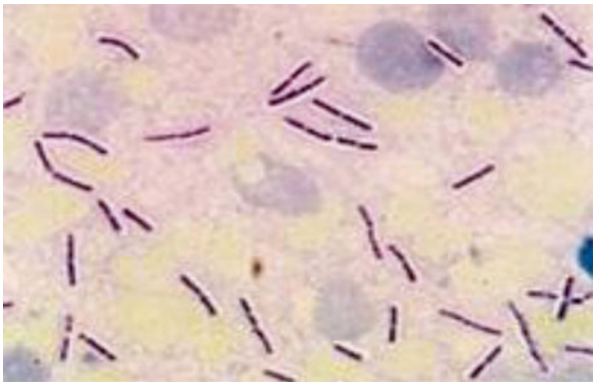


Fig. 8.9 *Bacillus anthracis* as large Gram-positive rod

Fig. 8.10 Sterile disposable cotton swab stick

A zigzag pattern can be used for wounds larger than 5 cm^2. This technique is likely to increase the yield. If pus or discrete abscesses are collected locally, the fluid should be aspirated into a syringe using a needle. The fluid is an ideal specimen for culture.

Cultures, while essential in the assessment of the microbiology of leg/foot infections, do not in isolation establish the presence of infection.

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